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Imidazoline Receptors: From Basic Concepts to Recent Developments

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Summary: The existence is now fully demonstrated of binding sites specifically recognizing the imidazoline structure or similar chemical structures, both in the brain and in certain peripheral tissues, including the kidney, some of which participate in the control of blood pressure. These binding sites are different from α_2-adrenoceptors, both functionally and biochemically. The fact that at least medullary binding sites are associated with a precise function, i.e., modulation of sympathetic activity of central origin and therefore the regulation of vasomotor tone, indicates that they are true receptors. These imidazoline receptors of the ventrolateral region of the medulla play an important role in the mechanism of the hypotensive action of substances such as clonidine or rilmenidine. Confirmation of dual pharmacological mechanisms involved in the hypotensive and sedative effects, respectively, of these drugs has justified attribution of the hypotensive effect to involvement of imidazoline receptors in the region of the medulla mentioned and the sedative effect to activation of classic α_2-adrenoceptors of the locus ceruleus. This distinction between two mechanisms opens up a novel approach to the development of new centrally acting antihypertensive substances free of the troublesome adverse effects of the first generation. Many questions remain unanswered, including the precise nature of the endogenous ligand(s) of imidazoline receptors, the structure of the receptor and its mechanisms of coupling to second messengers, the possible existence of variants of these receptors, their mode of interaction with α-adrenoceptors, and identification of true subtypes of these imidazoline receptors. **Key Words:** Hypertension—Imidazolines—Catecholamines—Central nervous system.

Involvement of the central nervous system in cardiovascular regulation, possibly in dysfunctional anomalies associated with certain forms of hypertension, and its role as a site of impact of cardiovascular drugs are experiencing renewed interest regarding the group of centrally acting antihypertensive agents.

Years ago, we advanced the hypothesis that the central hypotensive action of clonidine and of related substances could not be totally explained by an interaction of these substances with α_2-adrenoceptors, as was suggested at the time (1). We proposed the existence of receptors specifically recognizing these chemical structures and not belonging to the major group of α-adrenoceptors. Because the site of action of these hypotensive substances had already been located in the nucleus reticularis lateralis of the ventrolateral region of the medulla (2) (Fig. 1), we suggested that such receptors should exist in this part of the brain (3). This suggestion was based on our earlier finding indicating that norepinephrine, the physiological ligand of adrenergic receptors, was not capable of reproducing the hypotensive effects of clonidine when it was injected directly into this ventrolateral region of the medulla (4).

A structure–activity relation study enabled us to show that when substances with a catecholamine or imidazoline structure, i.e., similar to the reference substance, were injected directly into the medulla of anesthetized animals, only imidazolines and related substances had hypotensive effects. No catecholamine or chemically analogous substance had any such effect (3) (Fig. 2). Even α-methyl norepinephrine, the most selective catecholamine of α_2-adrenoceptors, never had any effect on blood pressure when injected into this region. It was subsequently shown that no correlation could be established between the affinity of imidazoline substances for α-adrenoceptors and their ability to lower blood pressure when injected directly into the medulla (5). All of this experimental background clearly suggested the existence of receptors

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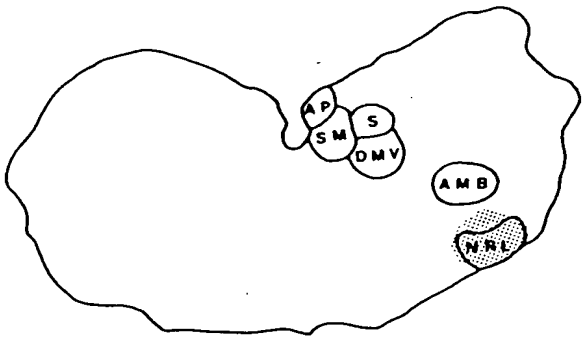

FIG. 1. Diagram of the frontal section of the medulla passing through the nucleus reticularis lateralis (NRL), area postrema (AP), nucleus ambiguus (AMB), dorsal motor nucleus of the vagus (DMV), S, and nucleus of the tractus solitarius (SM). Gray zone corresponds to the area of the medulla where imidazoline and analogous substances induce their hypotensive effect.

specifically sensitive to the imidazoline structure in the medulla, modulating sympathetic activity toward the periphery and therefore participating in blood pressure regulation.

In recent years, the existence of binding sites specific to imidazolines (receptors) has been shown in a wide variety of tissues and species (6–13). Attempts have been made in a number of laboratories throughout the world to purify this receptor and its endogenous ligand (14–18), and an arginine derivative, agmatine, has been suggested very recently as playing this role (19). Once the hypotensive action of imidazolines or analogues could be attributed to the involvement of non–adrenergic-specific receptors, it was legitimate to attempt to distinguish between the pharmacological effects involved in their therapeutic effect (hypotensive) and in their most common adverse effect (sedation), respectively.

The sedative effect of clonidine has been attributed to an action on a medullary structure different from

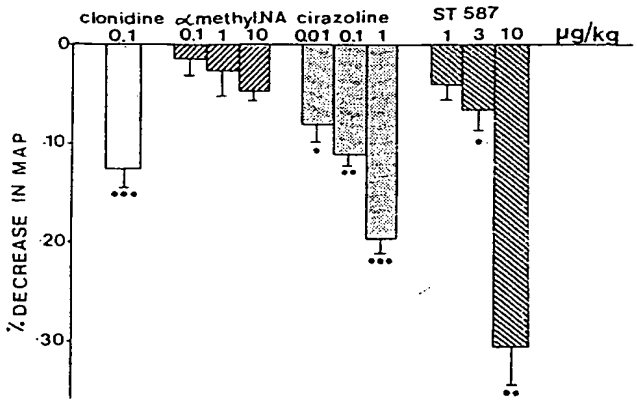

FIG. 2. Variations in mean arterial pressure (MAP) obtained after microinjection into the NRL of the anesthetized cat of several imidazolines and of a catecholamine (α-methylnorepinephrine, α-methyl NA).

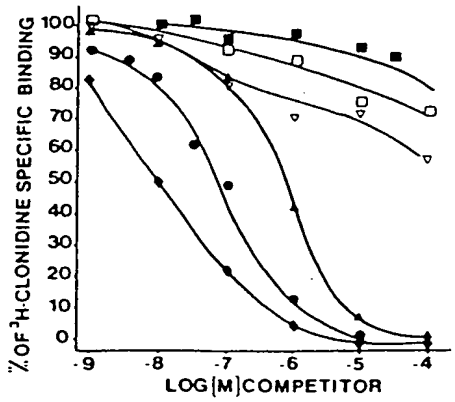

FIG. 3. Curves of displacement of specific binding of [^{3}H]-clonidine on neuronal membranes of the human NRL region by different imidazolines and catecholamines. ♦, Cirazoline; ●, clonidine; ▲, ST 587; ▽, α-methyl-NA; □, (−)epinephrine; ■, (−)norepinephrine.

that in which it induces its hypotensive action: the locus ceruleus. This dorsally located medullary nucleus is also part of the reticular formation and is very rich in noradrenergic neurons. It is directly involved in the regulation of wakefulness (20–22). Older studies had already shown that clonidine inhibited noradrenergic neurons in this medullary nucleus by activating α_2-adrenergic type receptors. This forms the basis of the explanation of its sedative effect (23–25).

BIOCHEMICAL CHARACTERIZATION OF BINDING SITES (RECEPTORS) SPECIFIC TO IMIDAZOLINES IN THE CENTRAL NERVOUS SYSTEM

The existence of specific imidazoline-binding sites, totally insensitive to catecholamines, in the central nervous system was reported for the first time by Ernsberger et al. in 1987 (26). They used *p*-aminoclonidine to label a subpopulation of binding sites in membrane preparations that were obtained from the ventrolateral region of bovine medulla and were totally insensitive to catecholamines. Even an excess of norepinephrine was unable to totally displace the radioactive ligand. The existence of these binding sites in the central nervous system has been confirmed since the use of tritiated clonidine in bovine and human brain (27–30) (Fig. 3), as well as tritiated idazoxan in the rabbit (7,31), guinea pig (11), and human (31–33).

Autoradiographic studies of the distribution of binding sites have revealed the existence, including in the human brain, of a specific distribution of these sites that is, at least in part, different from that of α_2-adrenoceptors (34,35). A number of other features sharply differentiate α_2-adrenoceptors, initially attributed with the hypotensive effect of clonidine-like substances, from imidazoline receptors. These include their insensitivity to GTP analogues, indicating non-

coupling to a G protein, whereas α_2-adrenoceptors clearly couple with a G protein (30,33,36). Several groups of researchers have also attempted to purify these binding sites and have shown them to consist of different protein entities from α_2-adrenoceptors (10,37–40).

Two of these attempts at purification have led to the isolation of a protein that could be a regulatory subunit of monoamine oxidase at mitochondrial level, for which imidazolines and related substances have a high affinity (see article by A. Parini in this volume), as well as to the isolation of a protein from human medulla that has all of the characteristics of an imidazoline-binding site and appears to be located on the neuronal plasmic membrane. This 43-kDa protein has every appearance of a neuronal receptor that could be involved in the hypotensive effect of these substances (41). A series of studies still must be undertaken to finally identify this protein and the gene coding for it. Such studies could lead to the identification of abnormal variants of this receptor protein, the involvement of which in certain forms of animal and human hypertension could then be sought.

FUNCTIONAL STUDIES

To confirm the hypothesis that specific imidazoline receptors might be particularly associated with the modulation and/or regulation of vasomotor tone, we undertook a series of in vivo experiments using the electrochemical technique differential voltammetry to study in parallel the effects of clonidine-like drugs on cardiovascular parameters and on the metabolic activity of catecholaminergic neurons of the medulla (42). This technique enables assessment of the overall activity of these neurons by measuring variations in the concentration in the extracellular medium of a catecholamine catabolite over the course of time. This concentration provides a reliable index of overall neuronal activity (43).

We were thus able to show that the reference substance, clonidine, at relatively low doses, markedly inhibited the neurons of the ventrolateral region of the medulla while simultaneously lowering blood pressure. We also found that the kinetics of these two effects were parallel (42). We described the existence of a positive correlation between hypotensive effects and neuronal inhibition effects seen in this region in relation to the doses of clonidine administered. These results indicated a very close relation between the two effects of clonidine with regard to the neurons of the ventrolateral region of the medulla.

However, when neuronal activity was measured in the locus ceruleus, which is where these substances produce their sedative effect, no evidence could be found of any such relation. Doses of clonidine 25 times greater than hypotensive doses were necessary to begin to inhibit the activity of these neurons. These studies thus provided evidence of a degree of regional specificity (42,44).

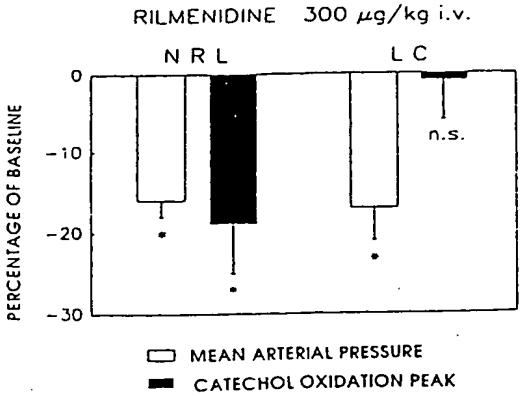

FIG. 4. Intravenous administration of rilmenidine in the anesthetized rat causes a fall in arterial pressure and inhibition of neuronal activity measured by the voltammetric peak of dihydroxyphenylacetic acid in the NRL, whereas neuronal activity in the locus ceruleus (LC) is unaffected.

Additional studies with selective antagonists led to definitive dissociation of the pharmacological actions involved in the hypotensive and sedative effects, respectively, of imidazolines and related substances (45). In other words, the origin of the hypotensive effect lies in the region of the nucleus reticularis lateralis and clearly involves imidazoline-specific receptors, whereas the origin of sedative effects lies in the locus ceruleus and no less clearly involves classic α_2-adrenoceptors.

RILMENIDINE

The structure of rilmenidine is similar to that of imidazolines. It is an oxazoline and is described as an antihypertensive substance, the action of which is in great part central, free of major adverse effects (46–48). In particular, it is free of sedative effects in laboratory experimental models, and the incidence of this adverse effect in humans at hypotensive doses also is very low (48,49). We were able to show that this substance has a pharmacological profile very similar to that of the reference substance, while being two to three times more selective for the region of the nucleus reticularis lateralis than for the region of the locus ceruleus. This finding emerged from a voltammetric study similar to that described (44) (Fig. 4). It is also striking to note that these functional studies have been confirmed by biochemical studies in which it has been shown that, in terms of affinity, rilmenidine was also two to three times more selective for imidazoline receptors in relation to classic α_2-adrenoceptors compared with clonidine (29). The hypotensive effect of this substance also is much more sensitive to the antagonistic effects of idazoxan, a selective antagonist of imidazoline receptors, than to those of yohimbine, a selective antagonist of α_2-adrenoceptors (18) (Fig. 5).

It therefore appears that in both animal models and humans, the absence of significant sedative effects

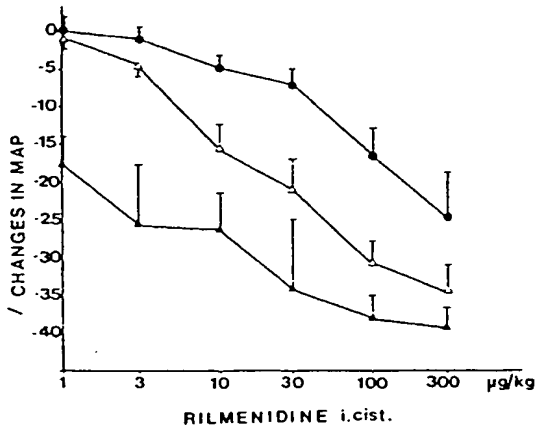

FIG. 5. Displacement of dose-response curves of rilmenidine (▲) administered directly in the cisterna magna by intracisternal injection of cumulative doses in the rabbit anesthetized with pentobarbital, as a result of pretreatment with idazoxan (●) and yohimbine (▽) administered via the same route.

could be attributable to a selectivity of substances for the imidazoline receptors of the ventrolateral region of the medulla. Rilmenidine thus is the first drug illustrating these concepts to emerge from our experimental research.

CLINICAL IMPLICATIONS

Imidazoline receptors are involved in the central hypotensive effects of clonidine-like substances and in the modulation of the regulation of vasomotor tone. A new generation of centrally acting antihypertensive agents has appeared, with significantly fewer adverse effects. In addition, studies of the renal effects of rilmenidine appear to indicate that substances with selectivity for imidazoline receptors in relation to α_2-adrenoceptors could possess an additional natriuretic action that, even if not major, could contribute to persistence in the long term of the antihypertensive effect of the substance and to the absence of water and sodium retention, as sometimes occurs during long-term treatment with the reference substance (6).

We have also suggested the hypothesis that dysfunction of the "imidazoline system" could be linked to the pathophysiology of certain forms of hypertension. It is conceivable that abnormalities concerning either the endogenous ligand or receptors themselves could contribute to the etiogenesis of certain forms of hypertension. We first directed our attention to abnormalities potentially affecting the endogenous mediator. Although the latter is as yet unidentified, we developed a strategy enabling evaluation of the hypothesis of an abnormality in the production of this mediator.

We developed polyclonal and monoclonal antibodies raised against clonidine used as immunogen by coupling it with a protein (50–53). Some of these antibodies proved to be highly specific to substances chemically close to clonidine while showing no evidence of crossover reaction with catecholamines or with other endogenous mediators that have a structure close to that of imidazolines (52). We chose this strategy because it has been shown for other systems that antibodies raised against exogenous ligands of specific receptors recognized the corresponding endogenous mediators. This had been reported in particular concerning anti-alprenolol (β-blocker) antibodies that specifically recognized endogenous catecholamines (54). Both polyclonal and monoclonal anti-clonidine antibodies used in radioimmunoassays enabled us to detect the existence of an immunoreactive substance in human serum. This study showed that its concentration, expressed in arbitrary units obtained from study calibration curves, could be as much as 75 units in normotensive subjects. Evaluation of a group of essential hypertensive patients showed that in approximately 30% of them, levels of the immunoreactive circulating substance were above the upper limit measured in normotensive subjects (as high as 400 units) (53).

Values measured in a preliminary series of secondary hypertensive patients (with pheochromocytomas and Conn's syndrome) were no higher than those seen in the control group. Nevertheless, this latter result must be taken only as an indication because the series is too small to justify any generalized extrapolation of these conclusions.

It would thus appear that a subgroup of patients with so-called essential hypertension may be found to have abnormally high circulating levels of the "imidazoline-like" immunoreactive substance. The question of whether this circulating immunoreactive substance is identical to the endogenous ligand of imidazoline receptors remains unanswered. Such an answer can be provided only by the purification and final identification of the two substances. It is extremely tempting to suggest a link between these high levels in certain hypertensive patients and hypertension itself. If such a link were to be confirmed, it would then be necessary to determine its nature, since at the moment there is nothing to show whether these high levels might be the cause of hypertension or whether they might be a possible consequence.

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